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Appl. No. 10/028,245
Amdt. dated October 24, 2005
Reply to Office action of June 15, 2005

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REMARKS

The Invention.

The presently claimed invention provides for a novel endoglucanase nucleic acid sequence, designated *egl8*, and the corresponding EGVIII amino acid sequence, as well as proteins having at least 95% identity thereto. The invention also provides expression vectors and host cells comprising a nucleic acid sequence encoding EGVIII and recombinant EGVIII proteins.

Status of the Application.

Claims 2, 4-17, 19-20, 22-24 and 26 are pending in the application. Applicants Claims 2 and 8 have been amended. Support for this amendment may be found throughout the specification as filed. Applicants assert new matter has not been introduced by the amendment.

Applicants thank the Examiner for the courtesies extended during the course of the telephonic interview held on October 24, 2005. As a result of the discussion, as well as the amendments made herein, the case is now thought to be allowable.

35 U.S.C. §112, first paragraph.

Claims 2, 5-17, 19-20 and 26 stand rejected under 35 USC §112, first paragraph as failing to be described in the specification. Specifically, the Examiner asserts that the claim scope is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of polynucleotides broadly encompassed by the claims. Applicants respectfully traverse.

Applicants have amended the claims to recite 95% sequence identity. In addition, the enzyme must possess endoglucanase activity. Therefore, the claims would encompass naturally occurring endoglucanases and variant endoglucanases that possess endoglucanase activity and have at least 95% sequence identity with EG VIII as defined in the present specification.

The first paragraph of 35 U.S.C. § 112 requires, *inter alia*, that the specification of a patent enable any person skilled in the art to which it pertains to make and use the claimed

invention. Although the statute does not say so, enablement requires that the specification teach those in the art to make and use the invention without 'undue experimentation.' In re Wands, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988). That some experimentation may be required is not fatal; the issue is whether the amount of experimentation required is 'undue.' Id. at 736-37, 8 USPQ2d at 1404. *In re Vaeck*, 947 F.2d 488, 495, 20 USPQ2d 1438, 1444 (Fed. Cir. 1991).

The question is whether the disclosure is sufficient to enable those skilled in the art to practice the claimed invention; the specification need not disclose what is well known in the art. Lindemann Maschinenfabrik GmbH v. American Hoist & Derrick Co., 730 F.2d 1452, 1463, 221 USPQ 481, 489 (Fed. Cir. 1984) (citing In re Myers, 410 F.2d 420, 161 USPQ 668 (CCPA 1969)). "A patent need not teach, and preferably omits, what is well known in the art." Spectra-Physics, Inc. v. Coherent, Inc., 827 F.2d 1524, 1534, 3 USPQ2d 1737, 1743 (Fed. Cir. 1987). "Not every last detail is to be described, else patent specifications would tum into production specifications, which they were never intended to be." In re Gay, 309 F.2d 769, 774, 135 USPQ 311, 316 (CCPA 1962).

Applicants assert that molecular biology techniques are well known and the skilled artisan is well aware of various techniques to alter polynucleotide sequences. Techniques for multiple amino acid substitutions that may be used, and were known at the time of filing, are methods of mutagenesis, recombination and/or shuffling followed by a relevant screening procedure, e.g., an assay such as those disclosed on page 11. Other methods that can be used include phage display (e.g., Lowman et al., Biochem. 30:10832-10837, 1991; Ladner et al., U.S. Pat. No. 5,223,409; Huse, WIPO Publication WO 92/06204) and region-directed mutagenesis (Derbyshire et al., Gene 46:145, 1986; Ner et al., DNA 7:127, 1988).

Mutagenesis/shuffling methods can be combined with high-throughput, automated screening methods to detect activity of cloned, mutagenized polypeptides in host cells. Mutagenized DNA molecules that encode active polypeptides can be recovered from the host cells and rapidly sequenced using modern equipment. These methods allow the rapid determination of the importance of individual amino acid residues in a polypeptide of interest, and can be applied to polypeptides of unknown structure.

If the Examiner believes that there is a lack of information on EG VIII Applicants would

like to draw the Examiner's attention to page 24 of the instant specification and Figure 2. Figure 2 provides the amino acid sequence of EG VIII. On page 24, Applicants provide information on homologous proteins and the glycosyl hydrolase family to which EG VIII belongs. Essential amino acids in the endoglucanase polypeptides of the present invention can be identified according to procedures that were *well known in the art at the time of filing*, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, Science 244: 1081-1085, 1989). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological activity (i.e., endoglucanase activity) to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton et al., J. Biol. Chem. 271:4699-4708, 1996. The active site of the enzyme or other biological interaction can also be determined by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids. See, for example, de Vos et al., Science 255:306-312, 1992; Smith et al., J. Mol. Biol. 224:899-904, 1992; Wlodaver et al., FEBS Lett. 309:59-64, 1992. The identities of essential amino acids can also be inferred from analysis of homologies with polypeptides which are related to EG VIII. Applicants have provided information on the protein structure of the enzyme and homologies with other polypeptides (see, for example, page 24 of the instant specification). Thus, one of skill in the art, at the time of filing, would have been able to design appropriate experiments to modify the EGVIII polynucleotide to produce a polypeptide having endoglucanase activity and having at least 95% sequence identity to SEQ ID NO:2 without Applicants having to provide every teaching within the four corners of the specification.

Direction of where to produce the alterations is provided by Applicants on pages 23 and 24. Depending on the characteristic to be altered, Applicants contemplated modifications directed to alteration of an active site, alteration of the pH optima, temperature optima, and/or substrate affinity of the EGVIII enzyme. Alignment with known family members would provide the skilled artisan with the appropriate starting point for modifications. The method of making the polynucleotides and polypeptides is not being claimed but it is enabled.

The Examiner asserts that the use of molecular modeling as provided for in the

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Mosimann et al. reference provided by Applicants is not generally applicable to cellulases because he has failed to find a single reference that cited Mosimann. However, the technique of comparing a first sequence with a second sequence and altering the first sequence is used routinely. It has been used on cellulases (see Sandgren et al., attached). In addition it has been applied to proteases (see, for example, WO 04/067737), phytases (see, for example, US 6391605) and pullulanases (see, for example, US 6838257). The technique has use in many different types of proteins, including cellulases.

Thus, the fact that Applicants do not explicitly provide examples regarding every polynucleotide encompassed by the present invention does not render the present claims unpatentable. The Specification teaches the DNA and protein sequence of EG VIII. Techniques were well known in the art on how to compare the protein with other proteins and have a reasonable expectation of success (see, for example, Mosimann et al., previously submitted and other materials provided herein), and for determining enzyme activity (e.g., using standard assays). Beginning with the sequence provided one of skill in the art would know how to proceed if they wanted to generate variants – compare the sequence with known related sequences, identifying in the three-dimensional structure at least one structural part of the parent cellulase; modifying the nucleic acid sequence encoding the parent cellulase to produce a nucleic add sequence encoding a variant of the parent cellulase having a deletion, insertion, or substitution of one or more amino acids at a position corresponding to said structural part; and expressing the modified nucleic acid sequence in a host cell to produce the variant. All of the methods and techniques were familiar to the skilled artisan and are not required to be taught in the specification. Thus, Applicants respectfully request that this rejection be withdrawn.

CONCLUSION

In light of the above amendments, as well as the remarks, the Applicants believe the pending claims are in condition for allowance and issuance of a formal Notice of Allowance at an early date is respectfully requested. If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (650) 846-7615.

Respectfully submitted,
GENENCOR INTL., INC.

Victoria L Boyd

Date: October 24, 2005

Victoria L. Boyd
Registration No. 43,510

Genencor International, Inc.
925 Page Mill Road
Palo Alto, CA 94304
Tel: 650-846-7615
Fax: 650-845-6504